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# Comparative mitogenomics of native European and alien Ponto-Caspian amphipods

Jan-Niklas Macher[1,2,3], Eglė Šidagytė-Copilas[2], Denis Copilaș-Ciocianu[2]

**1** *Naturalis Biodiversity Center, Darwinweg 2, 2333 CR, Leiden, Netherlands* **2** *Laboratory of Evolutionary Ecology of Hydrobionts, Nature Research Centre, Akademijos 2, 08412, Vilnius, Lithuania* **3** *Institute of Environmental Sciences (CML), Leiden University, Postbus 9518 2300 RA, Leiden, Netherlands*

Corresponding authors: Jan-Niklas Macher (jan.macher@naturalis.nl);
Denis Copilaș-Ciocianu (denis.copilas-ciocianu@gamtc.lt)

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## Abstract

European inland surface waters are home to a rich diversity of native amphipod crustaceans, many of which face threats from invasive Ponto-Caspian counterparts. In this study, we analyse mitochondrial genomes to deduce phylogenetic relationships and compare gene order and nucleotide composition between representative native European and invasive Ponto-Caspian taxa across five families, ten genera and 20 species (with 13 newly sequenced herein). We observe various gene rearrangement patterns in the phylogenetically diverse native species pool. *Pallaseopsis quadrispinosa* and *Synurella ambulans* exhibit notable deviations from the typical organisation, featuring extensive translocations of tRNAs and the nad1 gene, as well as a tRNA-F polarity switch in the latter. The monophyletic invasive Ponto-Caspian gammarids display a conserved gene order, primarily differing from native species by a tRNA-E and tRNA-R translocation, which reinforces previous findings. However, *Chaetogammarus warpachowskyi* shows extensive rearrangement with translocations of six tRNAs. The invasive corophiid, *Chelicorophium curvispinum*, maintains a highly conserved gene order despite its distant phylogenetic position. We also discover that native species have a significantly higher GC and lower AT content compared to invasive species. The mitogenomic differences observed between native and invasive amphipods warrant further investigation and could provide insights into the mechanisms underlying invasion success.

## Keywords

invasive, mitochondria, native, nucleotide composition, Ponto-Caspian, phylogeny

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## Introduction

The European continent harbours a vast diversity of inland amphipod crustaceans, found in surface or subterranean, fresh or brackish waters (Barnard and Barnard 1983; Väinölä et al. 2008; Borko et al. 2021; Copilaș-Ciocianu and Sidorov 2022). Moreover, this diversity is significantly underestimated due to the widespread prevalence of cryptic species (Copilaş-Ciocianu et al. 2018; Eme et al. 2018; Wattier et al. 2020; Bystřický et al. 2022). However, a substantial proportion of this fauna faces threats from invasive species, climate change, eutrophication and other anthropogenic factors (Fišer et al. 2010; Rewicz et al. 2014; Maximov 2021; Arbačiauskas et al. 2022). One of the main challenges native amphipods encounter is competition and potential extinction due to the spread of invasive counterparts, particularly those originating from the Ponto-Caspian Basin (Rewicz et al. 2014; Arbačiauskas et al. 2017; Copilaș-Ciocianu et al. 2023a; Dobrzycka-Krahel et al. 2023).

The Ponto-Caspian region encompasses the Azov, Black, Caspian and Aral seas, as well as the lower stretches of their tributaries (Copilaș-Ciocianu et al. 2023a). This area is characterised by a unique endemic fauna, particularly adapted to wide salinity fluctuations (Reid and Orlova 2002; Paiva et al. 2018). Due to their environmental tolerance, many Ponto-Caspian endemics have become invasive, expanding their range beyond native borders, mainly colonising European inland waters and even reaching other continents (Bij de Vaate et al. 2002; Copilaș-Ciocianu et al. 2023a). Amphipods represent one of the most successful groups, with up to 40% of the species pool spreading outside their native range during the last century, mainly due to increased shipping activity, construction of canals and intentional introductions (Arbačiauskas et al. 2011; Copilaș-Ciocianu et al. 2023a). Invasive Ponto-Caspian amphipods can be competitively superior to the native species they encounter along invasion routes, leading to the native species decline and eventual extinction (Dermott et al. 1998; Grabowski et al. 2007, 2009; Bacela-Spychalska and van der Velde 2013; Šidagytė and Arbačiauskas 2016; Minchin et al. 2019).

Comparative studies involving both native and invasive species are essential for understanding invasion success. However, the underlying molecular and genetic mechanisms behind the success of Ponto-Caspian species invading new areas are not well-known and research is still in its early stages (Adrian-Kalchhauser et al. 2020; Mamos et al. 2021). The mitochondrial genome is a good candidate for comparative molecular studies, as mitochondria are crucial for the functioning of multicellular life and complete mitochondrial genomes are relatively inexpensive and easy to sequence due to recent advances in high-throughput sequencing and bioinformatic pipelines (Trevisan et al. 2019; Macher et al. 2020). Given that studies generally reveal strong differentiation in respiratory function amongst native and invasive aquatic species (Lenz et al. 2011; Lagos et al. 2017a, 2017b; Hraoui et al. 2020), it is reasonable to assume that the structure of the mitochondrial genome may provide insights into invasion success.

To date, relatively few mitochondrial genomes are available for invasive Ponto-Caspian amphipods and native European species, many of which were obtained from transcriptomic data and are, thus, of varying reliability (Krebes and Bastrop 2012; Macher et al. 2017; Cormier et al. 2018; Bojko 2020; Mamos et al. 2021). Of the 13 widespread invasive Ponto-Caspian species, the mitochondrial genomic structure is reliably known for four species from two genera (*Dikerogammarus bispinosus*, *D. haemobaphes*, *D. villosus* and *Pontogammarus robustoides*), while the mitogenome of *Obesogammarus crassus* is only known from transcriptomic reads, resulting in some regions having low coverage and potentially reduced reliability (Mamos et al. 2021). With respect to native European species, the situation is more severe, as reliable mitogenomes are available for only four species out of several dozen (and possibly hundreds of species): *Gammarus duebeni*, *G. fossarum*, *G. lacustris* and *G. roeselii*. (Krebes and Bastrop 2012; Macher et al. 2017; Cormier et al. 2018; Sun et al. 2020), while four more species have mitogenomes assembled from transcriptomic reads (*G. pulex*, *G. wautieri*, *Echinogammarus berilloni* and *Pectenogammarus veneris*) (Cogne et al. 2019; Mamos et al. 2021). For the purpose of this study, we treat *G. roeselii* as a native south-east European species, although we acknowledge its non-native status in central-western Europe (Csapó et al. 2020).

In this study, we compare the mitochondrial gene order, nucleotide composition and assess the phylogenetic relationships of native European and invasive Ponto-Caspian amphipods. We present a significantly expanded dataset that includes mitochondrial genomes representing most major native and invasive species in Europe. We present the first mitochondrial genomes of native *Synurella ambulans*, *Pallaseopsis quadrispinosa*, *G. jazdzewskii* and *G. varsoviensis*, the first DNA-based mitogenome for *G. pulex* and the first mitogenome of *G. lacustris* from Europe (previously sequenced only from the Tibetan Plateau (Sun et al. 2020)). Regarding the invasive species, we present the first mitogenomes for *Chaetogammarus ischnus* and *C. warpachowskyi*, the first DNA-based mitogenome for *O. crassus* and additional mitogenomes for *D. haemobaphes*, *D. villosus* and *P. robustoides*. Lastly, we present the first mitogenome for *Chelicorophium curvispinum*, the most widespread Ponto-Caspian corophiid amphipod. Our study provides new insights into the mitochondrial genomes of native European and invasive Ponto-Caspian amphipods, enhancing our understanding of their phylogenetic relationships and potentially uncovering key factors contributing to the invasion success of these ecologically important species.

## Materials and methods

### Sampling, laboratory protocols and sequencing

Animals used in the analyses were collected from Lithuania, Poland and Latvia between 2018 and 2020 using kick-sampling with a hand net (see Suppl. material 1 for detailed locality information). Specimens were stored in 96% ethanol in the

field. Afterwards, the ethanol was exchanged several times and the material was stored at –20 °C. Specimens were identified using relevant keys (Eggers and Martens 2001; Copilaş-Ciocianu et al. 2014; Copilaș-Ciocianu and Sidorov 2022). The taxonomy of the focal taxa follows the most recent updates (Hou and Sket 2016; Sket and Hou 2018; Copilaș-Ciocianu and Sidorov 2022; Garcia-Paris et al. 2023; Horton et al. 2023).

We dissected the dorsal half of the animal (from head to urosome) using microsurgical scissors and fine needles to avoid contamination from the gut and extracted genomic DNA using the Quick-DNA Miniprep Plus Kit (Zymo Research) with the lysis step prolonged overnight. All specimens selected for high-throughput sequencing were also DNA-barcoded using the protocols described in Copilaş-Ciocianu et al. (2022) to further confirm morphological identifications.

After DNA extraction, we assessed quantity and fragment length of the genomic DNA using a FragmentAnalyzer (Agilent, USA). To fragment the DNA, the Covaris M220 system (Covaris, UK) was used targeting a fragment size of 250 base pairs. The fragmented DNA was then checked again on the FragmentAnalyzer system to confirm the quantity and length of fragments. The NEBNext Ultra II DNA Library Prep Kit and corresponding NEBNext Multiplex Oligos for Illumina were used to prepare shotgun genomic libraries following the manufacturer's protocol. The final library concentration and fragment size were confirmed on a TapeStation (Agilent) before manual equimolar pooling of samples. A negative control was processed together with the samples. It did not show any DNA when measured on FragmentAnalyzer and TapeStation before sequencing and was, therefore, not sequenced. The final library was sequenced using the Illumina NovaSeq 6000 platform with 2 × 150 bp read length at Macrogen Europe.

## Bioinformatics, mitochondrial genome assembly and annotation

Raw data were checked for low-quality samples using the FastQC software and Illumina adapters were trimmed using Trimmomatic (Bolger et al. 2014). Strict quality filtering was applied to trimmed reads using vsearch, with reads truncated at the first base with a phred score < 15. Reads shorter than 100 bp were excluded from subsequent analysis. Per sample, ten million quality-checked reads were assembled using Megahit (Li et al. 2015) on the Naturalis high-performance cluster, with kmer lengths ranging from 15 to 115. The resulting contigs were imported into Geneious Prime (v.2022.2) and BLAST searches were conducted against a manually compiled reference library of amphipod mitochondrial genes (Macher et al. 2017; Mamos et al. 2021) downloaded from NCBI GenBank (https://www.ncbi.nlm.nih.gov/genbank/). Contigs were identified as potential mitochondria, based on BLAST results and contig lengths (between 10,000 and 20,000 bp). Potential mitochondrial contigs were subsequently annotated using Mitos2 (Donath et al. 2019). Annotations were manually checked and refined in Geneious Prime and gene sequences (nucleotide and protein) were extracted for subsequent phylogenetic analyses.

## Nucleotide composition

We added the 13 mitogenomes obtained in this study to seven mitogenomes from previous studies, totalling 20 species, of which eight were Ponto-Caspian invaders and 12 native species (Table 1). Nucleotide composition was calculated for the entire mitogenomes using MEGA 6 (Tamura et al. 2013). To visualise patterns of composition amongst species, the percentage matrix of each of the four nucleotides was subjected to a Principal Component Analysis (PCA) using a variance-covariance matrix. A Permutational Multivariate Analysis of Variance (PERMANOVA) test with 9,999 permutations was used to detect differences in nucleotide composition between the native and invasive species groups. Furthermore, GC and AT content were separately compared between native and invasive species using a Mann-Whitney test. All analyses were conducted with PAST 4.10 (Hammer et al. 2001).

**Table 1.** Overview of the species used in the comparative analyses.

| Family | Species | NCBI accession number | Status | Mitogenome length (bp) | A % | T % | G % | C % | Source |
|---|---|---|---|---|---|---|---|---|---|
| Corophiidae | *Chelicorophium curvispinum*[1] | CC6 | Invasive | 14867 | 37.8 | 30.6 | 12.6 | 19.1 | This study |
| Gammaridae | *Chaetogammarus warpachowskyi*[1] | CW4 | Invasive | 17336 | 35.2 | 35.9 | 10.9 | 18.0 | This study |
| Gammaridae | *Chaetogammarus ischnus*[1] | EI4 | Invasive | 14694 | 32.5 | 33.9 | 12.1 | 21.5 | This study |
| Gammaridae | *Dikerogammarus bispinosus* | OK173840 | Invasive | 15336 | 33.9 | 36.6 | 11.1 | 18.3 | Mamos et al. (2021) |
| Gammaridae | *Dikerogammarus haemobaphes*[1] | DH3 | Invasive | 15258 | 31.9 | 34.2 | 13.1 | 20.9 | This study* |
| Gammaridae | *Dikerogammarus villosus*[1] | DV4 | Invasive | 15176 | 32.7 | 35.1 | 12.3 | 19.9 | This study* |
| Pontogammaridae | *Obesogammarus crassus*[1] | OC4 | Invasive | 15838 | 33.6 | 37.5 | 11.3 | 17.6 | This study† |
| Pontogammaridae | *Pontogammarus robustoides*[1] | PR4 | Invasive | 15917 | 33.3 | 36.2 | 11.8 | 18.7 | This study* |
| Crangonyctidae | *Synurella ambulans* | SA1 | Native | 15652 | 32.2 | 30.8 | 13.3 | 23.6 | This study |
| Gammaridae | *Gammarus duebeni* | JN704067 | Native | 15651 | 32.5 | 22.0 | 31.5 | 14.0 | Krebes and Bastrop (2012) |
| Gammaridae | *Gammarus fossarum* | KY197961 | Native | 15989 | 32.0 | 22.0 | 33.2 | 12.9 | Macher et al. (2017) |
| Gammaridae | *Gammarus lacustris* | GL1 | Native | 18195 | 31.1 | 32.8 | 13.3 | 22.8 | This study* |
| Gammaridae | *Gammarus jazdzewskii* | GZ1 | Native | 16136 | 34.6 | 34.4 | 11.4 | 19.5 | This study |
| Gammaridae | *Gammarus pulex* | GP2 | Native | 14886 | 33.1 | 34.0 | 12.2 | 20.7 | This study† |
| Gammaridae | *Gammarus roeselii* | MG779536 | Native/ Non-native | 16073 | 33.9 | 32.9 | 12.3 | 20.9 | Cormier et al. (2018) |
| Gammaridae | *Gammarus varsoviensis* | GV1 | Native | 15482 | 31.1 | 32.8 | 13.2 | 22.8 | This study |
| Gammaridae | *Gammarus wautieri* | BK059229 | Native | 13927 | 32.4 | 22.2 | 34.2 | 11.2 | Cogne et al. (2019), Mamos et al. (2021)† |
| Gammaridae | *Echinogammarus berilloni* | BK059223 | Native | 14454 | 30.2 | 26.9 | 28.0 | 14.9 | Cogne et al. (2019), Mamos et al. (2021)† |
| Gammaridae | *Pectenogammarus veneris* | BK059233 | Native | 14369 | 34.1 | 22.2 | 31.4 | 12.4 | Cogne et al. (2019), Mamos et al. (2021)† |
| Pallaseidae | *Pallaseopsis quadrispinosa* | PQ1 | Native | 16147 | 30.9 | 30.9 | 15.3 | 22.9 | This study |

[1] – Ponto-Caspian species; * – species whose mitogenomes were also sequenced in previous studies; † – species whose mitogenomes were previously assembled from RNA sequences.

### Phylogenetic analyses

The purpose of these analyses was to place the focal taxa within the broader phylogenetic context of Amphipoda. In total, the data obtained in this study were combined with an additional 62 mitogenomes from literature, representing 25 families and 59 species, including one isopod outgroup, *Ligia oceanica* (see Suppl. material 1 for further details). The analyses were based on the 13 protein-coding genes and excluded the large (16S rRNA) and small (12S rRNA) ribosomal subunits. Protein-coding genes evolve in a more predictable manner than the erratic ribosomal units and can be confidently aligned. Each of the 13 genes was aligned separately by codon using MUSCLE (Edgar 2004) implemented in MEGA 6 with default options. All nucleotide alignments were protein translated using the invertebrate mitochondrial genetic code (translation table 5). Individual gene alignments were concatenated using SequenceMatrix (Vaidya et al. 2011). Both nucleotide and translated protein sequences were used in the phylogenetic analyses. The concatenated nucleotide matrix had a total length of 11,047 bp, while the protein matrix was 3,682 amino acids long. The best partitioning schemes (by codon) and evolutionary models for the nucleotide data were selected with PartitionFinder 2 (Lanfear et al. 2016).

Phylogenetic analyses were conducted within a Bayesian (BI) framework with Phylobayes MPI 1.8c (Lartillot et al. 2013) and a maximum-likelihood (ML) framework with IQ-Tree 2.1.2 (Nguyen et al. 2015). Phylobayes nucleotide analyses were run for 10000 cycles using the GTR exchange rates and the CAT profile mixture. Convergence, mixing and effective samples size were checked by examining the relative differences amongst chains (< 0.2), as well as using Tracer 1.7 (Rambaut et al. 2018). IQ-Tree nucleotide analyses were run under an edge-linked model with each partition having an independent evolutionary model selected with PartitionFinder 2. Node support was assessed using 1000 ultrafast bootstrap replicates (Hoang et al. 2018). The protein phylogenetic analyses were run with the general metazoan mitochondrial amino acid substitution model (MtZOA) (Rota-Stabelli et al. 2009) for both Phylobayes and IQ-tree with the same settings as for nucleotides. All phylogenetic analyses were carried out using the computational infrastructure available at the CIPRES Science Gateway (Miller et al. 2010).

### Data accessibility

All mitochondrial genomes are available in NCBI GenBank, accession numbers OR233270–OR233282, as well as on Figshare (DOI: 10.6084/m9.figshare.22753487).

## Results

### Mitochondrial genomic structure

All samples yielded high-quality reads that could be assembled into complete mitochondrial genomes containing the expected number of 13 protein-coding genes, large and small-subunit rRNA and 22 transfer RNAs. Mitogenome length varied

between 14,694 bp (*C. ischnus*) and 18,195 bp (*G lacustris*); see Table 1 for length of all mitogenomes. In most cases, the inferred gene order is similar between the native and invasive Ponto-Caspian species. The most observed difference is a translocation of the tRNA-E and tRNA-R, which aligns with previous observations, based on less extensive taxonomic datasets (Bojko 2020; Mamos et al. 2021). However, there are also a few rather contrasting patterns of variation between the native and invasive groups (Fig. 1). The native species exhibit three general patterns: (1) minor translocations (swaps) between tRNAs (tRNA-N, tRNA-E and tRNA-R) as observed in *G. roeselii* and *G. varsoviensis*; (2) significant translocations of multiple tRNAs and the NADH dehydrogenase 1 gene (nad1) in *P. quadrispinosa* and *S. ambulans*; (3) a polarity switch of the tRNA-F in *S. ambulans*. The gene arrangement in the Ponto-Caspian gammarids is identical in all studied species, except *C. warpachowskyi*, which shows a significant departure with the translocation of six tRNAs. The gene order in the Ponto-Caspian alien corophiid *C. curvispinum* is identical to that of most native species (Fig. 1). In general, the gene arrangements appear to follow phylogenetic relationships.

## Nucleotide composition

Multivariate analyses indicate a significant differentiation with respect to nucleotide composition between the native and invasive species. The PCA scatterplot indicates a modest overlap between native and invasive groups in multivariate space, with the first two axes explaining 98% of the observed variance (Fig. 2A). The separation is further confirmed by PERMANOVA testing which indicates significant differences between the two groups ($F = 6.257$, $p = 0.01$). The invasive species are generally associated with a higher AT content, while native species with a higher GC content, although with a large variation in GC content of native species, is observed. Further univariate comparisons using Mann-Whitney tests reveal that invasive species have a significantly higher AT and significantly lower GC content than the native species (mean $AT\%_{invasive} = 68.86$, mean $AT\%_{native} = 60.99$, $z = 3.04$, $p = 0.001$; mean $GC\%_{invasive} = 31.15$, mean $GC\%_{native} = 38.97$, $z = 3.48$, $p = 0.001$) (Fig. 2B).

## Phylogenetic analyses

Phylogenetic analyses revealed congruent relationships between methods (BI and ML) and datasets (nucleotides and amino acids) (Fig. 3). Disagreements were observed only at unsupported nodes. The native European inland water species are phylogenetically diverse, interspersed between two main superfamilies, the Gammaroidea and Crangonyctoidea. Although the alien Ponto-Caspian species also belong to two main superfamilies, Gammaroidea and Corophioidea, the gammarids form a strongly-supported monophylum. Our analyses reveal for the first time the phylogenetic position of *P. quadrispinosa*, confirming it as a sister species to the Baikal endemic *P. kessleri* and ultimately part of the Baikal Lake acanthogammarid radiation.

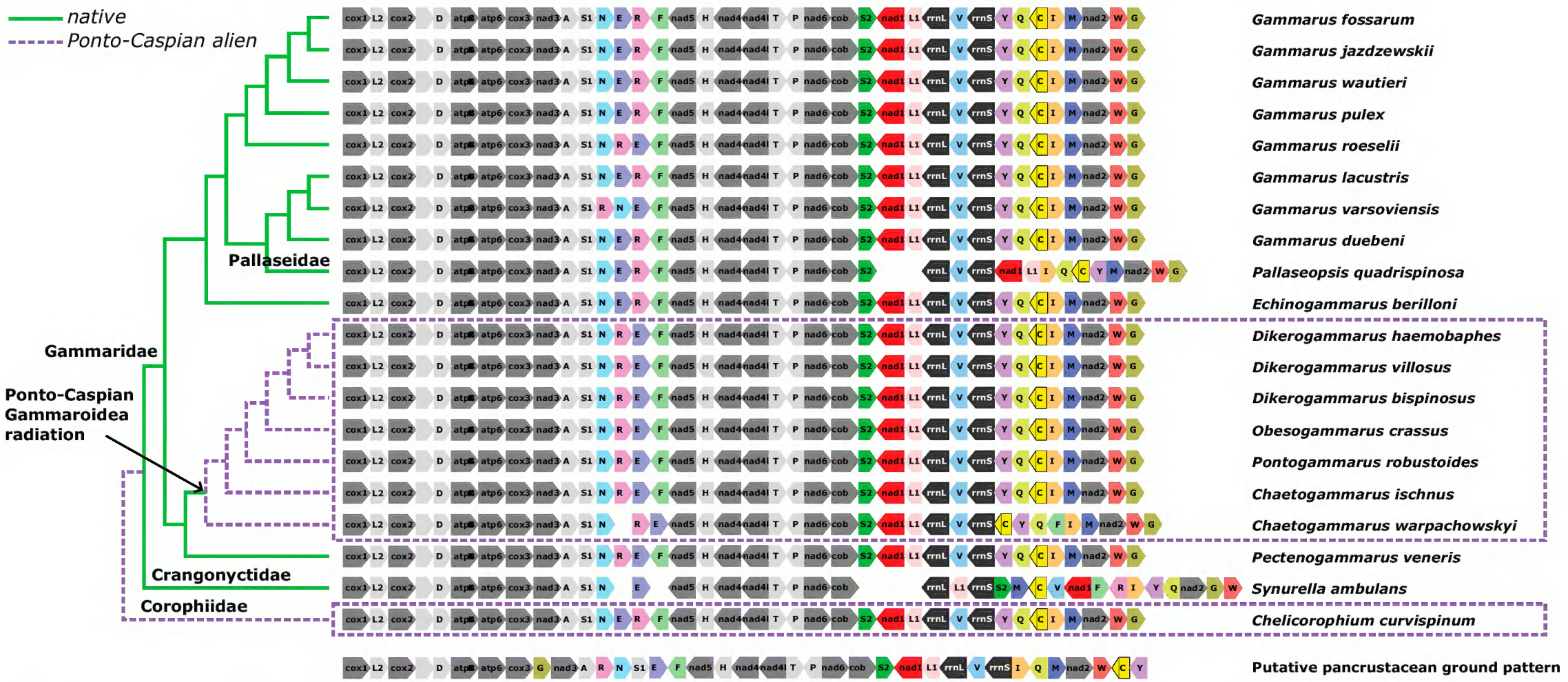


**Figure 1.** Comparison of mitochondrial genome organisation amongst focal species. Genes with conserved positions are shown in greyscale, translocated genes are coloured. Genes encoded on the plus-strand point towards the right, while the ones encoded on the minus-strand point towards the left. Invasive Ponto-Caspian species are shown with dashed lines. The cladogram on the left represents a summary of phylogenetic relationships from Fig. 3. The putative pancrustacean ground pattern is adapted from Stokkan et al. (2016).

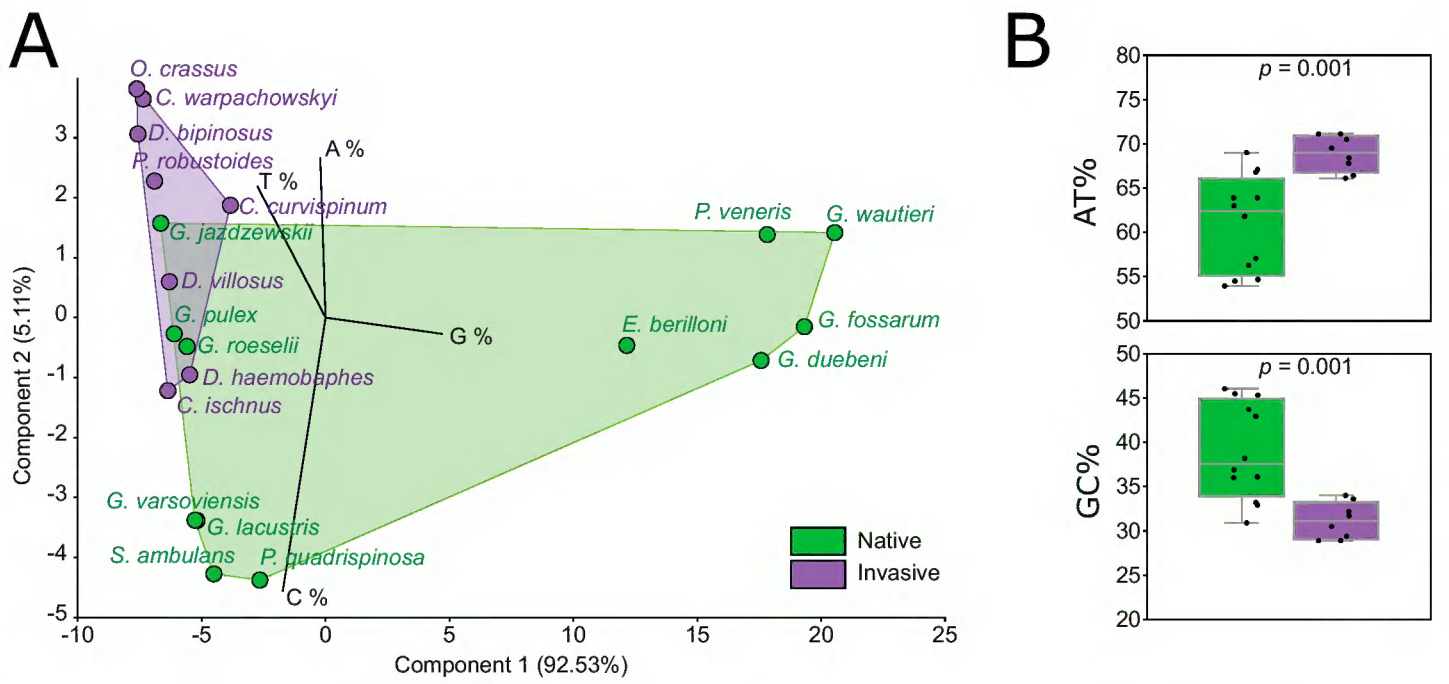


**Figure 2.** Differentiation of native European and invasive Ponto-Caspian amphipod species with respect to nucleotide composition across the entire mitochondrial genome. A) PCA scatterplot depicting multivariate differentiation across all four nucleotides; B) boxplots comparing AT and GC content between native and invasive species.

## Discussion

The patterns of mitogenomic rearrangements observed in this study are consistent with the diversity that has been observed in other amphipod clades, ranging from major differentiation at generic levels to highly conserved between divergent clades (Bauzà-Ribot et al. 2009; Stokkan et al. 2016; Li et al. 2019; Zapelloni et al. 2021). The mitogenomic phylogenetic relationships obtained herein are also in agreement with other phylogenetic studies, based on nuclear and mitochondrial markers (Copilaş-Ciocianu et al. 2020).

Our study reveals that the native inland European amphipods can exhibit substantial differences with respect to mitogenomic organisation, while the alien Ponto-Caspian species are more conservative. This is not unexpected given the greater phylogenetic disparity amongst the native species. However, the organisation patterns seem not always to be phylogeny driven. For example, *C. curvispinum*, which is distantly related to the other focal species in this study, exhibits a conserved gene arrangement, identical to that of most native species. On the other hand, *P. quadrispinosa* is more closely related to other native gammarids, yet it diverges significantly with respect to gene order. In fact, the gene order of *P. quadrispinosa* is identical to that of its congener from Lake Baikal, *P. kessleri* (Romanova et al. 2016). Our study confirms for the first time with molecular data the phylogenetic position of this species, which is a glacial relict that has almost become extinct in Central Europe due to climate warming and eutrophication (Meßner and Zettler 2021; Arbačiauskas et al. 2022). The peculiar mitogenomic structure of *Pallaseopsis* is outstanding even amongst other Baikalian amphipods (Rivarola-Duarte et al. 2014; Romanova et al. 2014, 2016, 2021), possibly reflecting intense periods of selection (Naumenko et al. 2017; Romanova and Sherbakov 2019).

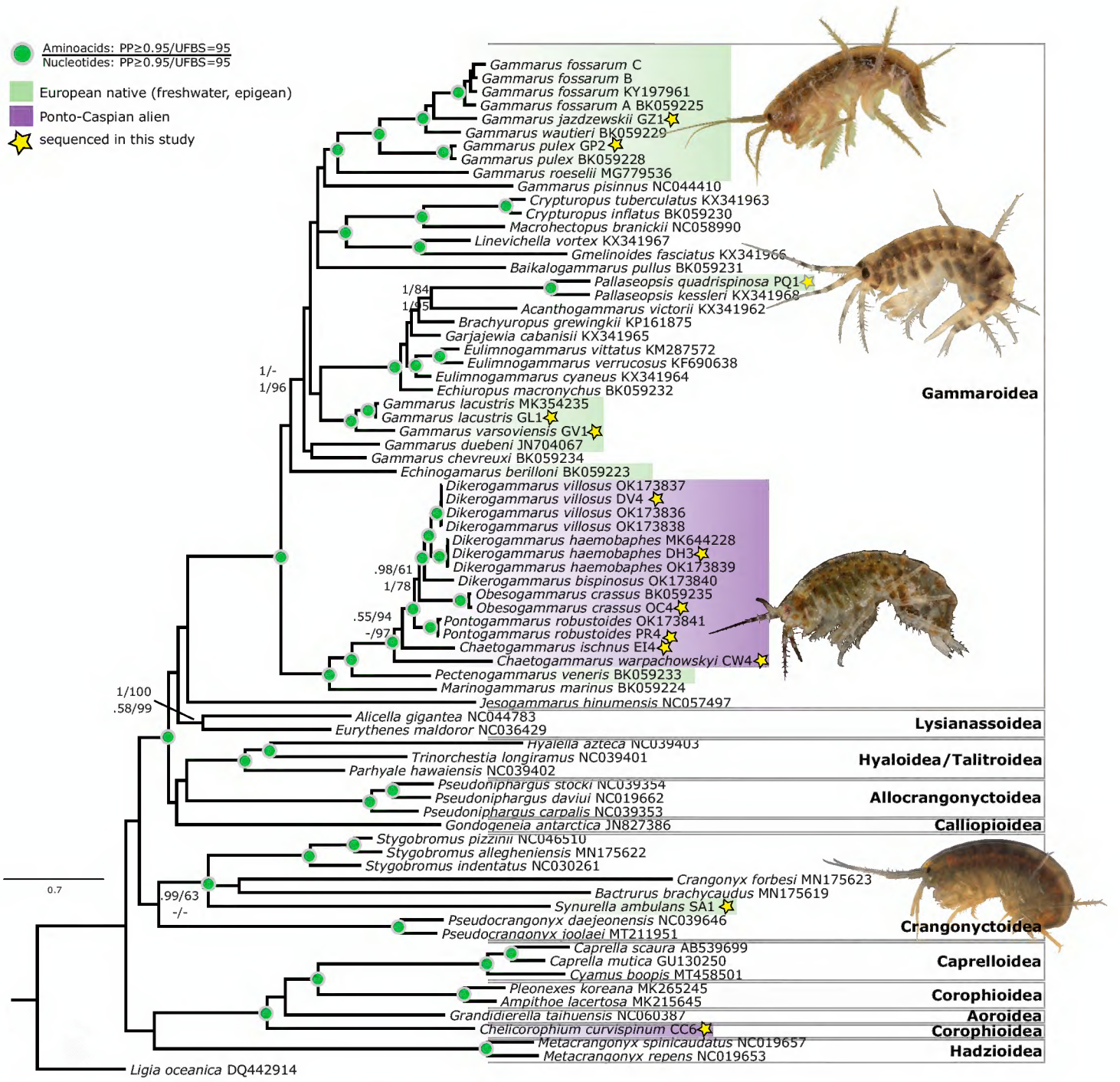


**Figure 3.** Amino acid Bayesian phylogeny, based on 13 mitochondrial protein-coding genes depicting the evolutionary relationships amongst the focal taxa (highlighted with colour). Native European surface-dwelling species are shown with green shading, while invasive Ponto-Caspian species are in purple. Stars indicate taxa sequenced in this study. Green circles indicate nodes that received strong support in all analyses. Nodes with numbers received moderate to strong support. Numbers above nodes indicate statistical support (posterior probabilities—PP; ultrafast bootstrap—UFBS) for amino acid-based trees; below nodes for nucleotide-based trees. Nodes that are not annotated received weak/no support (PP < 0.5, UFBS < 50%). Inset photographs from top to bottom: *G. fossarum*, *P. quadrispinosa*, *C. warpachowskyi* and *S. ambulans* (D. Copilaș-Ciocianu).

The native crangonyctid *S. ambulans* is phylogenetically very distant from the native gammarids and its mitogenomic structure is highly distinct as well. Several tRNAs and the nad1 gene in *S. ambulans* have undergone translocations. Moreover, we detected a switch to a positive polarity of the tRNA-F gene, which normally is found on the minus-strand in amphipods. This pattern is partially phylogeny-driven, because the available mitogenomes of other crangonyctids seem to be generally conserved, but in some cases can show significant transpositions (Benito et al. 2021). The remaining native gammarids

(*Echinogammarus*, *Gammarus* and *Pectenogammarus*) possess a conserved mitogenomic structure, with the main differences involving minor translocations of tRNAs, particularly between tRNA-E and tRNA-R. The mitogenome of *G. varsoviensis* exhibits a previously-unknown translocation of the tRNA-N, situated between tRNA-R and tRNA-E.

The alien Ponto-Caspian gammarids exhibit a more conserved gene order than their native counterparts. Apart from the phylogenetically distant *C. warpachowskyi*, all species have identical mitogenomic structures. They differ from native species due to a swap between tRNA-E and tRNA-R, a pattern observed in previous studies with less taxonomically comprehensive datasets (Bojko 2020; Mamos et al. 2021). However, we demonstrate that this does not apply to all Ponto-Caspian gammarids, as *C. warpachowskyi* exhibits significant differences from this pattern with translocations of six tRNAs. This deviation may be attributable to its phylogenetic position, since this species is more distantly related to other Ponto-Caspian gammarids and should be assigned to a new genus (Copilaş-Ciocianu et al. 2022; Copilaș-Ciocianu et al. 2023b). Sequencing additional mitogenomes from Ponto-Caspian gammaroidean species will likely uncover further gene rearrangement patterns, as only seven of 82 species have been sequenced so far (Copilaș-Ciocianu and Sidorov 2022).

Aside from gene order, we discovered substantial differentiation in nucleotide composition between native and invasive Ponto-Caspian species. Invasive species possess significantly more AT-rich mitogenomes than natives, while natives exhibit higher GC content. This finding suggests that invasive species may have longer non-coding regions or that native species have protein-coding genes with higher GC content, which overall indicates more compact mitogenomes in the latter (Romanova et al. 2020; Sun et al. 2022). The relationship between this differentiation in GC content and invasion ability remains unclear, but it could potentially open new avenues for research.

With respect to phylogenetic relationships, our study is in broad agreement with previous molecular work. We further confirm the phylogenetic disparity of the native species pool, mirroring previous multilocus phylogenies (Copilaş-Ciocianu et al. 2020). Specifically, we corroborate the polyphyly of the genus *Gammarus* by recovering the two main Baikal gammarid radiations (Acanthogammaridae and Micruropodidae+Macrohectopidae) as nested within it (Hou and Sket 2016; Romanova et al. 2016; Naumenko et al. 2017; Mamos et al. 2021) and, for the first time, confirm with molecular data that *P. quadrispinosa* is indeed of Baikalian origin. Our trees also show that crangonyctids are more distantly related to gammarids than the current morphology-based classification suggests (Lowry and Myers 2017). Regarding the invasive Ponto-Caspian species, we confirm the monophyly of the gammaroids (Hou et al. 2014) and reveal for the first time the position of *C. curvispinum*.

## Conclusion

Our comparative analyses highlight substantial differentiation between the mitogenomes of native European and invasive Ponto-Caspian amphipod crustacean species. Native species, being more phylogenetically diverse, display varied mitogenomic

configurations and higher GC content compared to the less phylogenetically dispersed invasive species, which exhibit highly conserved gene order and increased AT content. We propose that these differences are not solely determined by phylogeny, as gene order conservation can vary across phylogenetic depths, but may also be shaped by other evolutionary factors including selective pressure. Exploring the biological implications of these mitogenomic distinctions between native and invasive amphipods may provide insight into the adaptive mechanisms that contribute to invasion success.

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## Supplementary material 1

**Origin of samples and mitochondrial genomes used in this study**

Authors: Jan-Niklas Macher, Eglė Šidagytė-Copilas, Denis Copilaș-Ciocianu

Data type: table (excel file)

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